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Review on Bovine Tuberculosis

Bemrew Admassu, Elias Kebede and Anmaw Shite

Department of Veterinary Pharmacy and Biomedical Sciences,
Faculty of Veterinary Medicine, University of Gondar, Gondar, Ethiopia, P.O. Box 196

Abstract: Bovine tuberculosis (BTB) is a chronic infectious disease caused by *Mycobacterium bovis*. This disease is widely distributed throughout the world and mainly affects animals with occasional human involvement. BTB can have an impact on the national and international economy, affects the ecosystem via transmission to wildlife and is of public health concern due to its zoonotic potential. It is primarily of economic importance as it can have a considerable direct effect on milk and meat production and animal reproduction. Although still present in some industrialized countries, BTB today mostly affects developing countries lacking the resources to apply expensive test and slaughter schemes. In Africa, the disease is present virtually on the whole continent; however, little accurate information on its distribution and prevalence is available. It is a chronic, generally respiratory disease, which is clinically difficult to diagnose although emaciation, loss of appetite, chronic cough and other signs of pneumonia could be symptoms developing at relatively late stages of the infection in cattle. Its pathology is characterized by the formation of granulomatous lesions, which can within the course of the disease regress or exhibit extensive necrosis, calcify or liquefy and subsequently lead to cavity formation. During meat inspection procedures on cattle carcasses in slaughterhouses, tuberculous lesions are primarily found in the upper and lower respiratory tract and associated lymph nodes. However, the bacteria can also develop a systemic infection, disseminate within its host and affect other organs. Aerosol exposure to *M. bovis* is considered to be the most frequent route of infection in cattle, but infection by ingestion of contaminated material may also occur. However, *M. bovis* infection in humans can occur through the consumption of contaminated raw or undercooked dairy and/or meat products; meanwhile occupational infection may occur due to exposure through airborne infection among farmers, veterinarians and slaughterhouse workers. Identification of *M. bovis* by culture, molecular techniques and biochemical methods is important for definitive diagnosis. Evaluations of antemortem tests for the diagnosis of BTB in Africa are scarce but a prerequisite to identify appropriate tools for future disease control programs. Control and prevention of the disease is vaccination and proper management of animals and humans environment.

Key words: Bovine tuberculosis · Mycobacterium bovis · Epidemiology · Diagnosis · Prevention and Control

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by mycobacteria that has been a major health risk to human and animals for more than a century. It is widely distributed throughout the world affecting all age groups of humans and animals. In humans, it is responsible for more deaths than any other bacterial disease ever today. Bovine tuberculosis (BTB) is a disease characterized by formation of granulomatous nodules called tubercles whose locations depend largely on the route of infection. In calves, it is usually transmitted by ingestion and lesions involve the mesenteric lymph nodes with possible spread to other organs [1]. In older cattle, infection is usually by the respiratory tract with lesions in the lung and dependent lymph nodes [2].

Tuberculosis is a chronic infectious disease of animals, birds and humans, caused by members of the genus *Mycobacterium*. In most species it can lead, with the proliferation of tubercles, to caseation and

Corresponding Author: Bemrew Admassu, Department Veterinary Pharmacy and Biomedical Sciences, Faculty of Veterinary Medicine, University of Gondar, Ethiopia.
Tel: +2519342489. Skype name: Thomas.admassu2. E-mail: vet27admasu@gmail.com.

calcification in the parenchyma of affected organs [3]. Signs like emaciation and weakness are just two of the cardinal alert symptoms during ante mortem inspection, though these two alone do not confirm bovine tuberculosis [4].

Mycobacterium bovis is the major causative agent of bovine tuberculosis (BTB) and part of the Mycobacterium tuberculosis complex (MTBC). Although still present in some industrialized countries, BTB today mostly affects developing countries lacking the resources to apply expensive test and slaughter schemes. In Africa, the disease is present virtually on the whole continent; however, little accurate information on its distribution and prevalence is available [5].

Invisible droplets (Aerosols) containing TB bacteria may be exhaled or coughed out by infected animals and then inhaled by susceptible animals or humans. The risk of exposure is greatest in enclosed areas, such as barns. Inhalation of aerosols is the most common route of infection for farm and ranch workers and veterinarians who work with diseased livestock. Livestock also are more likely to infect each other when they share a common watering place contaminated with saliva and other discharges from infected animals [6]. Both beef and dairy cattle are susceptible to bovine TB. However, confinement dairies and feedlots are the primary areas of concern, frequent herd additions increase the opportunity for introduction of the disease and high animal densities increase the likelihood of the disease spreading among herd mates. However, because dairy cattle remain in the herd longer and are maintained in relatively dense populations, dairies are at higher risk for heavy herd infection rates [7].

It is also a known cause of zoonotic tuberculosis in humans, which can appear indistinguishable with regard to pathogenesis, lesions and clinical findings to that caused by *M. tuberculosis*. *M. bovis* shows a high degree of virulence for both humans and animals [8].

On a global scale, this zoonotic pathogen is estimated to cause 10–15% of human TB cases in the developing world [8] and is considered to be the fourth most significant livestock disease in terms of impact on human health and economics in developing countries, including risks to other livestock and wildlife [9]. Furthermore, there is evidence that subclinical BTB has a negative impact on productivity in dairy cows, increasing the costs incurred by the dairy industry [10]. As many parts of the world have no active surveillance programmes and limited epidemiological studies, the prevalence and impact of BTB worldwide is likely to be underestimated [11].

The risk of BTB to cattle is dependent on host, pathogen and environmental factors and there is a broad spectrum of outcomes to infection with *M. bovis* [12, 13] which are thought to be similar to the effects of the related pathogen *M. tuberculosis* Waters *et al.* [14] in humans.

The culling loss due to the disease is estimated to be 30-50% of the difference between the values of a dairy or beef breeding cow and its value at slaughter [15]. Consumption of raw or unpasteurized animal products and contact with infected carcasses plays a large role in zoonotic M. bovis infection of humans in Africa and South America [16].

The occurrence of BTB due to *M. bovis* in humans is difficult to determine accurately because of technical problems in isolating the microorganism. Currently, the BTB in humans is becoming increasingly important in developing countries, as humans and animals are sharing the same micro-environment and dwelling premises, especially in rural areas. At present, due to the association of mycobacterium with the *HIV/AIDS* pandemic and in view of the high prevalence of *HIV/AIDS* in the developing world and susceptibility of *AIDS* patients to tuberculosis in general, the situation changing is most likely. Prevalence data on BTB infection in Africa is scarce. There is, however, sufficient evidence to indicate that it is widely distributed in almost all African countries and even is found at high prevalence in some animal populations [17].

Tuberculosis due to *M. bovis* is still a neglected disease in animals as well as in human populations in the sub-Saharan countries, where it is less studied [6]. In addition to these bottlenecks to the development of the livestock industry, the disease attains much of its importance from being zoonotic, causing human tuberculosis. In humans TB is still a major cause of death worldwide in general and in the high-burden regions in particular [18-20].

In Ethiopia*, M. bovis* infection is endemic in cattle [21]. The prevalence of BTB in Ethiopia ranged from 3.4% in smallholder production systems to 50% in intensive dairy production system [22, 23]. Moreover, a prevalence of 5.15% of BTB was reported in animals slaughtered in Nazareth municipality abattoir of central Ethiopia [24]. Ethiopia, animals are kept in the same dwelling with their owners and use of dungs for plastering of wall, floor and as a source of energy for cooking, doexacerbate chances of spreading the disease human [25]. Thus, it is endemic and has been reported in different regions of the countries, the disease in the countries associated with decreased productive efficiency and carcass or organ

condemnation in the abattoir, the nationwide distribution of the disease and the economic loss associated with it has not been fully determined due to lack of good diagnostic facilities. Hence, having the knowledge of distribution, prevalence and risk factors of the disease are fundamental so as to look for effective control strategy [25].

Though detection of BTB in Ethiopia is most commonly carried out on the basis of tuberculin skin testing and abattoir inspection [26] regular surveillance through skin testing of millions of individual cattle, bacteriology and molecular methods are not realistic methods for logistic reasons. Abattoir inspection at the moment remains economically affordable and valuable techniques to detect TB lesions in the carcass of slaughtered cattle. The main purpose of post mortem examination of carcasses at slaughtered house is for the protection of the public health, but failure of detecting a lesion during inspection in cattle with a single lesion will have a huge zoonotic implications. So it is imperative to evaluate the efficiency of the routine and detailed meat inspection for the detection of suggestive tuberculosis lesions [27]. Therefore, the aim this review paper provides tips of information on Bovine tuberculosis so that proper control/preventive measures could be put in place.

General Characteristics of *Mycobacterium Bovis* and Its Host Range: *Mycobacterium bovis* is the organism that causes bovine tuberculosis. The bacteria are acid fast, filamentous, curved rods [28]. The organism does not grow on blood agar plates and requires6-8 weeks of incubation time to see visible growth on Lowenstein-Jensen media. Acid fast staining would yield acid fast positive rod shaped organisms on sputum smears. The tubercle bacillus is about as susceptible to the action of heat and light as any other vegetative organism; but is highly resistant to the action of chemical substances, a fact made use of in obtaining pure cultures from contaminated pathological material. Though drying kills a fair proportion of tubercle bacilli, many may escape this effect. The organism can also survive for long periods in cool shady places, particularly if protected from light by crust formation on infective discharges or dung [29].

Mycobacterium bovis, causative agent of BTB, is a member of the Mycobacterium tuberculosis complex, which also comprises the important human pathogen M. tuberculosis, as well as Mycobacterium canettii, Mycobacterium africanum, Mycobacterium pinnipedii, Mycobacterium microti and Mycobacterium caprae. These phylogenetically closely related bacteria share more than 99.9% chromosomal identity and they cause tuberculosis with similar pathology in various mammalian hosts [30].

M. bovis shows a dysgonic colony shape on Lowenstein-Jensen medium, is negative for niacin accumulation and nitrate reduction, is susceptible to thiophene-2-carboxylicacid hydrazide (TCH) and shows microaerophilic growth on Lebek medium. A further criterion used for differentiation is the intrinsic resistance to pyrazinamide, which is found in most *M. bovis* isolates. In contrast, *M. tuberculosis* shows eugonic growth, is positive for niacin accumulation and nitrate reduction, is resistant to TCH, shows aerophilic growth on Lebek medium and is usually not monoresistant to pyrazinamide More recently, several molecular methods have been developed that provide clear criteria for the identification of *M. bovis* [31].

Historically, taxonomic segregation of the *M. tuberculosis* complex has been based on each species' unique combination of host preference and its characteristic growth, morphology, physiology and biochemistry [32, 33]. No other TB organism has as great a host range as bovine TB, which can infect all warm blooded vertebrates. *M. avium* can affect all species of birds, as well as hogs and cattle. M. tuberculosis primarily affects humans but can also be transmitted to hogs, cattle and dogs. Bovine TB has affected animal and human health since antiquity. Once the most prevalent infectious disease of cattle and swine in the United States, bovine TB caused more losses among U.S. farm animals in the early part of this century than all other infectious diseases combined [34].

Susceptible host: *M. bovis* is of significant importance in livestock and in a wide range of wild animal species worldwide. Bovine species, including bison and buffaloes, are particularly susceptible to the disease, but nearly all warm-blooded animals can be affected. *M. bovis* is also known to affect humans, causing a serious public health problem where the disease is endemic [9]. Cattle are the usual host for *M. bovis*, but bovine TB can be transmitted to humans as well as other animals such as swine, bison and cervids (Deer and elk) [35].

Epidemiology

Source of Infection and Modes of Transmission: *M. bovis* infection is spread to cattle primarily through the inhalation of infectious aerosols, but has also been reported to be spread by ingestion of infectious material from drinking infected milk or ingesting contaminated pasture or feed. But inhalation is the most likely and

important route of infection in cattle with TB, since lesions in field cases predominantly involved the upper and lower respiratory tract and associated lymph nodes [36]. Cutaneous, congenital and genital infections have been recorded but are considered rare. Carrier animals are significant in spreading and perpetuating the infection, but transmission is intermittent and mimics a point source epidemic. Aerosol transmission occurs in all environments and the infective dose by inhalation can be very low. However, transmission is only effective over short distances, of 1–2 meter and cattle density is therefore a significant factor in the rate of transmission. Infection is spread more rapidly in intensive animal husbandry situations than in extensive or rangeland conditions [37].

Even in developed countries, clusters of cases of *M. bovis* TB have been associated with eating under processed or soft cheeses produced in countries with high rates of bovine TB. Humans can also become infected through inhalation of infectious aerosols and through direct exposure of cuts and abrasions (Known as 'butcher's wart'). Laboratory-derived cases of *M. bovis* TB have been recorded and infection has been reported in patients with human immunodeficiency virus HIV [38]. Reports of person-to-person spread of *M. bovis* infection are rare, strengthening the belief that *M. bovis* is not as infective for humans as *M. tuberculosis* [37, 39].

As is also true of human TB, the risk of *M. bovis* infection in humans is likely to increase where the prevalence of HIV/AIDS is high due to the susceptibility of immunosuppressed AIDS patients to TB. Cases of HIV-related human TB due to *M. bovis* have been reported in many developed countries. The potential impact of AIDS/HIV infections in humans on the transmission of *M. bovis* to and among humans is of great concern and requires careful consideration, wherever bovine TB is still a major problem [40].

Incubation Period: The incubation period can range from months to years with the severity depending on the immunity of the host, the size and frequency of the infectious dose and host genetics. In many cases, infection will be localized and cleared by the immune system, such that disease never develops. In humans, only 10% of people infected with *M. tuberculosis* will develop TB disease in their lifetimes [37]. Infection results in chronic disease; animals typically present with clinical signs during times of increased stress or as they age [41]. Persistence of the Agent.

M. bovis is an obligate intracellular parasite and has a limited survival period outside the host (Depending on the environmental conditions). It is susceptible to drying and ultraviolet light, but is relatively resistant to detergents and moderate changes in P^H. Bovine tuberculosis (BTB) is a persistent problem among UK cattle herds. Potential obstacles to BTB control are the existence of the badger (Meles meles) as a wildlife reservoir [42] and the presence of M. bovis in the environment where the organism can survive for months and may remain infectious. Badgers form social groups that use communal underground sets where conditions are likely to facilitate transmission and provide one focus of environmental contamination [43].

The local persistence of infection within small populations, such as cattle herds, depends on the maintenance of unbroken chains of transmission or the import of infection from a source(s) external to the herd. Regardless of its original source, infection can persist in cattle herds despite regular tuberculin testing and this facilitates amplification within herds and cattle-cattle spread. It is important to consider the extent to which cattle-cattle transmission, whether driven by susceptibility or not, contributes to maintenance (Persistence) of infection and what measures could mitigate or minimize the risk of further transmission [44].

Epidemiological Factors Influencing Transmission: Consistent with the mostly aerosol spread of *M. bovis*, disease prevalence is higher under intensive farming practices, such as on dairy farms or where animals are housed indoors. In beef herds, prevalence will generally be lower, but high prevalence (Around 35%) has been observed where cattle are overstocked and/or in poor condition. In very extensive farming systems, such as in pastoral cattle management, herd prevalence will generally be lower than in intensive systems, but small family groups can have high prevalence. Under pastoral conditions in northern Australia, opportunities for transmission were provided by cattle congregating around waterholes during the dry season, or on reduced amounts of dry land during the wet season [37].

The pre-disposing causes that might come into play are listed below: disease of civilization / domestication: - herding together facilitates spread; Age: young animals are more susceptible than older ones; Nutrition: Vitamin A & C deficiency predisposes; Housing: Dark, ill ventilated, damp dwellings are favorable for the spread;

Heredity: Zebu cattle are somewhat more resistant than exotic or crossbred; Climate: Cold and humid weather is favorable for spread [29].

Geographical Distribution: The geographical distribution of bovine TB has changed drastically over the past decades. Prior to the introduction of control measures and milk pasteurization in developed countries, TB has been widely distributed throughout the world. Eradication programmes based on test-and-slaughter policies to clear herds of infected animals virtually eliminated TB from livestock in many developed countries. Today, many countries in Europe and North America and Australia are free of the disease or close to complete eradication in livestock. However, the maintenance of *M. bovis* infection by wildlife species has compromised eradication efforts in countries such as in the United Kingdom, Ireland, New Zealand and parts of the United States of America [12]. In developed countries, the driving forces for the control and eradication of bovine tuberculosis from the national domestic herd are indisputably of economic and sociopolitical nature, based mainly on the negative economic impact of the disease [45]. In large parts of the developed world, policies regulating the control of bovine tuberculosis are aimed at complete eradication of the disease from its livestock populations as part of an integrated approach to food safety. These policies follow an expensive test-and slaughter strategy for the control of bovine tuberculosis and significant successes have been achieved in many countries [45, 46]. On the other hand, the benefit and sustainability of such costly programmes have been increasingly questioned in the light of the rising economic burden and social impacts on and reduced acceptance by farmers [47, 48]. However, in general, with the exception of a few countries with a wildlife reservoir of M. bovis the prevalence of bovine tuberculosis has reached very low levels, in most developed countries [48].

In developing countries, data on the prevalence of bovine TB are minimal and the information available may not represent the true epidemiological status of the disease. Although bovine TB is notable in many countries, it is often underreported, particularly in countries that lack effective disease surveillance and reporting systems. The insidious nature of the disease, which does not cause fulminating outbreaks with high mortality, is likely to decrease reporting of the disease, leading to a lack of measures for its control [49].

Despite disease under-reporting in developing countries, there is, however, sufficient evidence to indicate that not only the prevalence of disease is higher in the developing nations but also that in the absence of any national control and eradication programmes, it is increasing worldwide particularly in Africa [17], Asia and Latin America [12].

In general, the situation is profoundly different in developing countries, which are in general unable to apply expensive test-and-slaughter schemes for the control of animal tuberculosis. Although in parts of the Latin American and Caribbean countries there has been significant progress in bovine tuberculosis control and infection rates under 1% have been reported for 30% of the region's cattle, 70% of cattle are kept in areas where rates of infection are higher and where herd prevalence of up to 56% have been reported [50]. On the African continent, more than 80% of the human population co-exists with cattle in the absence of any organized control of bovine tuberculosis [51]. In recent years a growing awareness of neglected zoonoses including bovine tuberculosis has led to initiative supported by the WHO/FAO/OIE to investigate, calculate and mitigate the unknown risk from these animal diseases on livestock productivity, human health and livelihoods [52]. Overall, the presence and extent of bovine tuberculosis in the developing world has been poorly investigated in the past, but a number of recent studies have revealed new data confirming the presence of M. bovis in cattle [53-56] and moreover providing insights into the specific risk factors associated with tuberculosis in cattle in different countries and regions. In Africa, high prevalenc rates of bovine tuberculosis (up to 50% at herd level) were reported in areas of Zambia where cattle and Kafue lechwe shared grazing and water as well as in areas where the traditional management of livestock in transhumant herds (herds which are moved to floodplains for grazing during the dry season) prevailed [54, 57]. Under these often nomadic conditions, the risk of exposure to M. bovis was increased significantly by creating multiple herd contacts and increasing the total herd size. The latter has also been suggested as a driver of the disease prevalence in Ethiopia [58] and Ecuador [59]. On the other hand, in countries with a rapidly increasing livestock production and intensification of production systems such as Iran, the propagation and insufficient detection of circulating M. bovis strains may be the most important contributor to increasing economic losses from bovine tuberculosis,

rather than the importation of infected cattle, as previously suggested [60]. Most importantly, in the mainly rural livestock producing areas of developing countries, bovine tuberculosis can have devastating impacts on the livelihood of millions of the world's most vulnerable communities as the disease compromises their sustainable food supply, income and social status [54, 60].

Pathogenesis: After inhalation, the most part of *bacilli* are arrested in the upper respiratory tract. The bacilli which reach to alveoli will be ingested by alveolar macrophages. Tubercle bacilli withstand phagocytosis (Due to a lot amount of lipids into the cell wall) and multiply in the macrophages. Accumulating *mycobacterium* stimulate an inflammatory focus and cell-mediated hypersensitivity. Activated macrophages release cytokines which are responsible for specific tissue lesion, named tubercle. Tubercle is an avascular granuloma, composed of a central zone with giant cells and peripheral zone with lymphocytes and fibroblasts (Epithelioid cells) [61]. The ability of *mycobacteria* to survive and multiply within macrophages determines whether disease will occur within the host. Survival and multiplication of the organisms in macrophages at primary site of infection happens due to prevention of phagosome-lysosome fusion [62].

Pathogenicity of mycobacteria depends on their ability to escape phagocytic killing, mostly imparted by the cell wall constituents: Cord factor (Trehalose dimycolate), surface glycolipid responsible for serpentine growth in vitro; Suphatides, surface glycolipid containing sulphur which prevents fusion of phagosome with lysosome and cAMP secreted by the bacteria may also facilitate this; LAM heteropolysaccharide which inhibits macrophage activation by IFNã and induces macrophages to secrete TNFá which induces fever and IL-10 which suppresses mycobacteria-induced T cell proliferation; the wax of the cell wall, peptidoglycans and other glycolipids are responsible for the adjuvant activity attracts antigen presenting cells [63].

Mycobacteria are released from macrophages and also migrate within macrophages around the body. Waxy cell wall contributes to the host immune response to the mycobacteria and the development of lesions. The host mounts a cell-mediated immune response with activated macrophages and sensitised T cells followed by a delayed-type hypersensitivity response with granuloma formation [64].

Many lipids have been implicated in mycobacterial virulence which is not found in other bacterial genera. Lipomannan (LM), lipoarabinomannan (LAM), the phosphatidylinositol mannosides (PIMs), the cord factors trehalose mono- and dimycolate (TMM and TDM) and the phthiocerol dimycocerosates (PDIMs) are all surface bound mycobacterial lipids capable of modulating innate immunity [62]. Recently, newly identified lipids, such as monomycolyl glycerol (MMG), have been shown to modulate host immunity [65] and hyper virulence [66].

The organism replicates intracellularly after it has been taken up by the macrophages. A granuloma or tubercle forms as the body tries to wall off the infected macrophages with fibrous tissue. The granuloma is usually 1-3 cm in diameter, yellow or gray, round and firm. On cut section, the core of the granuloma consists of dry yellow, caseous, or necrotic cellular debris. The infection can spread hematogenously to lymph nodes and other areas of the body and cause smaller, 2-3 mm in diameter, tubercles. The formation of these smaller tubercles is known as "Miliary tuberculosis" [28].

Clinical Signs: Mycobacterium bovis is considered to be a slow growing microbe with a doubling time of around sixteen hours. This may account for why the host which is infected could take months to show the effects of the infection. TB caused by this strain of Mycobacterium can cause different symptoms depending on where the infection is taking place. If the infection is in the GI tract for example a main symptoms is extreme abominable discomfort, an infection in the lungs causes an almost uncontrollable cough. It is has been believed that the symptoms can be more closely related to the genome of this strain than the species. Mycobacteriums all produce mycolic acids in their cell walls. These acids could cause the symptoms seen from the infection of M. bovis [67].

When present, clinical signs can include variable pyrexia, weakness, anorexia, emaciation, dyspnea, enlargement of lymph nodes and coughing, particularly with advanced TB. These signs are not unique to bovine TB. Although normally a chronic debilitating disease, bovine TB can assume a more acute, rapidly progressive course [7, 68].

Diagnosis of the Disease: Many methods exist to diagnose cattle suffering from tuberculosis. Among them, In vivo test like tuberculin test, the cellular test based on the quantification of gamma interferon, post mortem

diagnosis of macroscopic lesions of tuberculosis, microscopic examination of lesion, culture, biochemical characterization, enzyme linked immunosorbent assay (ELISA) testing, tuberculin testing can be performed and molecular methods [69]. The various symptoms that are observed to be present can all help in diagnosing tuberculosis in cattle [68].

Gross and Histopathology: TB causes abscess-like lesions commonly referred to as granulomas or tubercles. The area of the body affected is usually related to the route of entry. Because of the frequency of respiratory transfer, lesions are often seen in the lungs and associated lymph nodes. Macroscopic lung lesions are not essential for the spread of TB by the respiratory route. Small and even microscopic lung lesions, which often occur concurrently with thoracic lymph node lesions, are often not detected by normal abattoir or field autopsy techniques [28].

Once the organism has entered the bloodstream, lesions may be found in any part of the body and may result in animals with 'generalized TB. The detection of macroscopic lesions at necropsy is an important aspect of the diagnosis of bovine TB. A presumptive diagnosis of bovine TB is often made on the basis of gross pathology and examination of smears or histological sections made from lesions. However, a definitive diagnosis can only be made by isolating *M. bovis* from animal specimens [70]. Lesions in cattle are most frequently seen at necropsy in the retropharyngeal, bronchial and mediastinal lymph nodes, which may be the only affected tissue. The lung, liver, spleen and the surface of body cavities may also be affected. Lesions in other species can differ from the classical picture seen in cattle [2].

Lesions in cattle may vary in size from 1 mm to more than 10 cm in diameter. There may be single lesions in lymph nodes or a primary complex that is, lesions in a parenchymatous organ and a lymph node draining the organ. Most lesions appear as firm or hard, white, grey or yellow nodules. The cut surface usually shows a yellowish, caseous centere, which is dry and firm [2]. Calcification is common, particularly in lymph nodes and on sectioning the lesion, a gritty sensation and grating sound indicate its occurrence. Conglomerate tubercles, formed by the growth and coalescence of one or more adjacent tubercles, may occur over the pleural or peritoneal surfaces. Metastases give rise to myriad tubercles of the same size, usually 2–3 mm in diameter. Old lesions may be encapsulated by connective tissue, heavily calcified and inspissated (Very dense) [28, 29, 41].

The histological lesions consist of necrotic cells in the center of the tubercle surrounded by epitheloid cells and multinucleated giant cells all encapsulated by collagenous connective tissue. The necrotic core of cells can often become calcified as the tubercle matures [28].

Culture of Mycobacterium

Media for Mycobacteria: The egg based Lowenstein-Jensen and stone brinks media are most commonly used in veterinary bacteriology. Lowenstein-Jensen medium can be obtained commercially. An agar-based medium such as middle brook is used by the bacteria to grow [69]. The media are prepared as solid slants in screw-capped bottles. Malachite green dye (0.025g/100ml) is commonly used as selective agent. *Mycobacterium tuberculosis*, *Mycobacterium avium* and many of the atypical *mycobacteria* require glycerol for growth. However, glycerol is inhibitory to *Mycobacterium bovis* while sodium pyruvate (0.4%) enhances its growth. Thus, themedia with glycerol and without glycerol (But with sodium pyruvate) should be inoculated. The mediacan be made more selective by the addition of cycloheximide (400ìg/ml), lincomycin (2ìg/ml) andnalidixic acid (35ìg/ml). Each new batch of culture medium should be inoculated with the stock strainsof *Mycobacteria* to ensure that the medium supports satisfactory growth [71]. The inoculated media may have to be incubated at 37°C for up to 8 weeks and preferably for 10 to 12 weeks with or without carbondioxide for the *mycobacteria* in the tuberculosis group [69]. *Mycobacterium tuberculosis* and *Mycobacteriumavium* prefer the caps on the culture media to be loose while *Mycobacterium bovis* grows best in air tight containers [70].

Colonial Morphology: The luxuriant growth of *Mycobacterium tuberculosis* on glycerol containing media, giving the characteristic 'Rough, tough and buff'colonies is known as eugenic while the growth of *Mycobacterium avium* on media containing glycerol isalso described as eugenic. *Mycobacterium bovis* has sparse, thin growth on glycerol containing media that is called dysgenic. *Mycobacterium bovis*, however, grow well on pyruvate-containing media without glycerol [72].

Histology and Acid-fast Staining: During necropsy of cattle suspected of being infected with BTB, tissue samples are collected and examined for histopathological (Microscopic) lesions that are compatible with *M. bovis*. In addition to looking for specific lesions under the microscope, pathologist can use special stain to identify

Table 1: Biochemical differentiation of *Mycobacteria* of tuberculosis group

Tests	*M. tuberculosis*	*M. bovis*	*M. avium*
Niacin production	+	_	_
Pyrazinamide production	+	_	+
Nitrate reduction	+	_	_
Inhibited by TCH 10mg/ml Resistant Susceptible Resistant	R	S	R
Urease	+	+	_

where, + = positive, _= negative, TCH= thiophen-2-carbonic acid hydrazide
Source: Quinn and Markey [70]

organisms that are compatible with *M. bovis*, the bacterium that causes BTB. The high lipid content, which ranges from 20 - 40% of the dry cell weight, is largely responsible for the ability of these bacteria to resist decolonization with acidified organic solvents [73]. The bacteria that take up this stain, including *M. bovis*, will appear as short red or pink rods when examined under a microscope [72].

Preliminary examination of tissues suspected of being tuberculous should include the preparation of suitably stained smears. The identifiable smear can be made on a new slide from scrapings of the cut surface of tissue. The smear should be air dried and fixed by flaming for one to two seconds. The kinyoum modification of the Zeihl-Neelsen stain is recommended because no heat is required [69]. The Zeihl-Neelsen method is commonly used to stain the mycobacteria. The smears are treated as with concentrated carbol fuchsin by heating and then decolorized with a sulfuric acid and alcohol solution. Malachite green or methylene blue is commonly used counter stains [73]. The stained slides are observed with an ordinary light microscope for the presence of acid-fast bacilli, which appear as red, colloidal or bacillary cells 1-3 microns in length occurring singly or in clumps [72].

Pigment Production and Response to Light: The *Mycobacteria* that produce yellowish-orange carotenoid pigments are called chromogenic [70, 74]. The term photo chromogenic is applied to those mycobacteria that produce pigment only if exposed to light. The scotochromogenic *Mycobacteria* produce pigment when incubated either in light or in the dark. Pigment formation is tested with young, well-developed colonies on Lowenstein-Jensen medium. The cultures are exposed to a 100 Watt, clear electric light bulb, at adistance of 50 cm, for at least an hour and then incubated again in darkness for a further 1-3 days. After this treatment the photochromogens will develop pigment. Older colonies of mycobacteria in the tuberculosis group often have a yellowish hue but they are described as non-chromogenic [70].

Biochemical Tests: The definitive identification of thespecies of mycobacteria is largely based on biochemical criteria [73]. These biochemical tests are niacin production test, nitrate reduction, deamination of pyrazinamide, urease test, inhibition and tolerance test [70].

Immunological Diagnostic Tests

Tuberculin Skin Test: The tuberculin test based on adelayed type hypersensitivity to mycobacterial is the standard ante mortem test in cattle. It is convenient, cost effective method for assessing cell mediated responses to a variety of antigens and it is “gold standard” for diagnostic screening for detection of new or asymptomatic Mycobacterium tuberculosis complex infection [75]. The reaction in cattle is usually detectable 30-50 days after infection. The injection site should be examined for a reaction 72 hours post inoculation [41]. The tuberculin is prepared from cultures of *M. tuberculosis* or *M. bovis* grown on synthetic media. The tuberculin test is usually performed between the mid necks, but the test can also be performed in the caudal fold of the tail. The skin of the neck is more sensitive to tuberculin than the skin of the caudal fold. To compensate for this difference, higher doses of tuberculin may be used in the caudal fold of the tail [69].

Bovine tuberculin is more potent and specific and the potency of tuberculins must be estimated by biological methods, based on comparison with standard tuberculins and potency is expressed in the international unit (IU) [2]. In several countries, bovine tuberculin is considered to be of acceptable potency if its estimated potency guarantees per bovine dose at least 2000 IU in cattle. In cattle with diminished allergic sensitivity, a higher dose of bovine tuberculin is needed and the volume of each injection dose must not exceed 0.2m. Cell mediated hypersensitivity, acquired through infection can be demonstrated systematically by fever or ophthalmically by conjunctivitis, or dermally by local swelling, when tuberculin test or its purified protein derivative (PPD) is given by the subcutaneous conjuctival or intradermal route, respectively [69].

Table 2: Comparison of tuberculin tests

Tests	Usage	Advantage	Disadvantage
Single intradermal test	Routine testing	Simple	Prone to false positive and Poor sensitivity
Comparative intradermal test	When avian TB or Johne's disease is prevalent	More specific than SID	More complex than SID
Short thermal test	Used in postpartum animal's and in infected animals	High efficiency	Time consuming and risk of anaphylaxis
Stormont test	Used in postpartum animal's and in advanced cases	Very sensitive and accurate	Three visits required May sensitize an animal

Source: Tizard [76]

Molecular Diagnosis of the Disease: Following preliminary screening of suspected samples using acid fast staining, isolation can be carried out in a bacteriological medium. However, cross-contamination among bovine carcasses, improper decontamination procedure and duration of isolation procedure (often 3 weeks and up to 8-10 weeks in liquid medium) jeopardizes the isolation of M. bovis. The lengthy duration of isolation procedure imposes an unavoidable delay in important decisions about outbreaks and of suspected herds put under restriction. Shorter time-span diagnostic procedures are required for quicker decision [69]. Therefore, there is an urgent need for a rapid, safe and reliable method to diagnose of bovine TB. The most promising technique for approaching this diagnostic dilemma is polymerase chain reaction (PCR). PCR has been used to amplify different regions of the mycobacterial genome, making it a good candidate for assisting with species identification in a variety of specimens [77].

Rapid identification of isolates to the level of M. tuberculosis complex can be made by Gen Probe TB complex DNA probe or polymerase chain reaction (PCR) targeting 16S–23S rRNA, the insertion sequences IS6110 and IS1081 and genes coding for M.-tuberculosis-complex-specific proteins, such as MPB70 and the 38 kDa antigen b have been used. Specific identification of an isolate as M. bovis can be made using PCR targeting a mutation at nucleotide positions 285 in the oxyR gene, 169 in the pncA gene, 675/756/1311/1410 and 1450 of the gyrB gene and presence/absence of RDs (Regions of Difference) [78]. Alternatively molecular typing techniques, such as spoligotyping will identify M. bovis isolates and provide some molecular-typing information on the isolate that is of epidemiological value [79].

PCR has been widely evaluated for the detection of M. tuberculosis complex in clinical samples (mainly sputum) in human patients and has recently been used for the diagnosis of tuberculosis in animals. A number of commercially available kits and various 'in-house' methods have been evaluated for the detection of the M. tuberculosis complex in fresh and fixed tissues [69]. Various primers have been used, as described above. Amplification products have been analysed by hybridisation with probes or by gel electrophoresis. Commercial kits and the in-house methods, in fresh, frozen or boric acid-preserved tissues, have shown variable and less than satisfactory results in interlaboratory comparisons [80]. False-positive and false negative results, particularly in specimens containing low numbers of bacilli, have reduced the reliability of this test. Variability in results has been attributed to the low copy number of the target sequence per bacillus combined with a low number of bacilli. Variability has also been attributed to decontamination methods, DNA extraction procedures, techniques for the elimination of polymerase enzyme inhibitors, internal and external controls and procedures for the prevention of cross-contamination. Improvement in the reliability of PCR as a practical test for the detection of M. tuberculosis complex in fresh clinical specimens will require the development of standardized and robust procedures [69]. Cross contamination is the greatest problem with this type of application and this is why proper controls have to be set up with each amplification. However, PCR is now being used on a routine basis in some laboratories to detect the M. tuberculosis group in paraffin embedded tissues [81, 82]. Although direct PCR can produce a rapid result, it is recommended that culture be used in parallel to confirm a viable M. bovis infection [82].

A variety of DNA-fingerprinting techniques has been developed to distinguish the M. tuberculosis complex isolates for epidemiological purposes. These methods can distinguish between different strains of M. bovis and will enable patterns of origin, transmission and spread of M. bovis to be described [83, 84]. The most widely used method is spoligotyping (from 'spacer oligotyping'), which allows the differentiation of strains inside each species belonging to the M. tuberculosis complex, including M. bovis and can also distinguish M. bovis

from M. tuberculosis [79]. The use of a standard nomenclature for the spoligotypes according to the database Mbovis.org (http://www.mbovis.org) is encouraged to allow international comparison of profiles.

Other techniques include restriction endonuclease analysis (REA) and restriction fragment length polymorphism (RFLP) using IS6110 probe (especially where there are >3–4 copies of IS6110 in the isolate), the direct repeat (DR) region probe, the PGRS (polymorphic GC repeat sequence) probe [85] and the Pucd probes [86]. The mycobacterial interspersed repetitive units (MIRU)-variable number tandem repeat (VNTR) typing has also been developed to increase the discrimination of the M. tuberculosis complex species [69]. Often a combination of techniques may be used to gain the maximum discrimination between strains [87].

The genome of M. bovis has been sequenced [88] and this information has contributed to improved methods of genetic fingerprinting and to the development of PCR assays that define the subspecies of the M. tuberculosis complex [69].

Economic and Public Health Importance of the Disease: The economic impact of bovine TB on livestock production is extremely difficult to determine accurately. The disease reduces livestock productivity in general and may be economically devastating for the cattle industry, especially the dairy sector [89]. Most important is the impact of the risk of infection to humans, particularly for women and children who appear to be more susceptible to the disease in countries with poor socio-economic conditions and weak veterinary and public health services. Although estimates of the costs associated with bovine TB and its control refer only to specific countries, all data suggest that worldwide economic losses due to the disease are significant. These losses include those related to animal production, markets and trade as well as the costs of implementating surveillance and control programmes. Losses to TB are also extremely important when endangered wildlife species are involved [89, 90].

In general BTB affects the national and international economy in different ways. The most obvious losses from BTB in cattle are direct productivity losses (Reduced benefit), which can be categorized into slaughter and “On-farm” losses. Slaughter losses comprise the cost of cattle condemnation and retention, with the loss from condemnation being essentially the purchased value of a slaughter animal and the loss from retention being a fraction of the value of a carcass. On-farm losses comprise the losses from decreased milk and meat production, the increased reproduction efforts and replacement costs for infected cattle [90].

Apart from direct productivity losses, BTB has profound economic consequences for national and international trade. On an international scale, BTB affects access to foreign markets due to import bans on animals and animal products from countries where the disease is enzootic. This situation has also major implications for other economic sectors, which are linked to livestock production. Moreover, BTB can create inefficiencies in the world market as e.g. economically inefficient but disease free exporting countries will receive more revenues than economically efficient countries, which cannot export animal products due to enzootic BTB [2]. Presence of the disease in wildlife has considerable economic consequences. Not only is disease eradication more difficult and costly but BTB can theoretically affect entire ecosystems with unpredictable impact on many areas of private interest such as e.g. tourism [90].

The economic impact of BTB in Africa is exacerbated through a number of factors. First, the fast growing population, especially in urban areas, causes an increase in demand especially for dairy products and meat and promotes the intensification of livestock production in peri-urban areas [90]. Importantly, intensive livestock production systems show generally a higher prevalence of BTB than extensive production systems. Second, developing countries lack the financial resources for disease control. This leads to a vicious cycle in which increased poverty affects the means for disease control and vice versa. Third, wildlife reservoirs in Africa are difficult to control; also, contact between transhumant cattle herds and wildlife may be particularly difficult to prevent in Africa. Forth, African countries have little access to the international trade and sanitary measures in industrialized countries may be used for protectionist purposes. Fifth, the public and political awareness are very low [89].

Bovine TB is a zoonotic disease that can have serious consequences for public health. The transmission of *M. bovis* from cattle to humans was once common in industrialized countries, but human infections were virtually eliminated in countries with effective programmes for eradicating the disease in cattle and high standards of food safety, particularly the pasteurization of milk. The incidence of human TB due to *M. bovis* varies considerably among countries depending on the prevalence of the disease in cattle, socio-economic conditions, consumer habits and practiced food hygiene.

In developed countries, *M. bovis* generally accounts for an in signicant share of total TB cases in humans. It, causes less than 2 percent of all TB cases in the United States of America [91] affecting primarily immigrant populations from Mexico and has been estimated to cause less than 1.5 percent of human TB cases in the United Kingdom [4]. In the Netherlands, *M. bovis* infection represented about 1.4 percent of all TB cases during the period of 1993 to 2007 [92].

In developing countries, the occurrence of human TB due to *M. bovis* is difficult to determine accurately and probably remains under-reported, owing to the diagnostic limitations of many laboratories in isolating the microorganism and distinguishing *M. bovis* from MTB [93]. Prevalence of the disease is likely to be higher in countries where *M. bovis* infection is endemic in cattle and milk is not pasteurized. Some reports have speculated that *M. bovis* accounts for 10 to 15 percent of human TB cases [94] while other estimates range from 0.4 to 8 percent, demonstrating that *M. bovis* is an important factor in human TB [40].

The proportion of which BTB contributes to the total of tuberculosis cases in humans depends on the prevalence of the disease in cattle, socioeconomic conditions, consumer habits, practiced food hygiene and medical prophylaxis measures. In countries where BTB in cattle is still highly prevalent, pasteurization is not widely practiced and/or milk hygiene is insufficient, usually estimated to be about 10% to 15% of human tuberculosis is considered to be caused by BTB [95].

Regassa [96] demonstrated the association of *M. tuberculosis* and *M. bovis* in causing tuberculosis between humans and cattle. The cattle owned by tuberculous patients had a higher prevalence (24.3%) than cattle owned by non-tuberculous owners with 8.6%. The author also noted that 73.8% and 16.7% of 42 human isolates were identified as *M. tuberculosis* and *M. bovis* and from cattle isolates 18.1% and 45.5% of 11 were found to be *M. tuberculosis* and *M. bovis* species, respectively. This showed that the role of *M. bovis* in causing human tuberculosis seemed to be significantly important. On the other hand, in Ethiopia, consuming raw meat is a welcome tradition, thus meat may also remain to be another area of concern or threat to be a source of BTB infection [96].

Control and Prevention: Control methods have served to reduce the number of infections. The rate of bovine tuberculosis in the United States is so low that the disease is considered to be practically eradicated. Solutions of phenol, iodine, glutaraldehyde and formaldehyde all have been found to be good disinfectants against the bacteria, as has exposure to heat of 250°Fahrenheit [97].

Vaccination for the prevention of bovine tuberculosis is another option that is being investigated. A vaccine was created for *M. bovis* in the 1920s by Calmette and Guerin. The vaccine reduces the severity of the disease, usually allowing the bacteria to infect only a few lymph nodes, but does not prevent infection. The goal is to develop an enhanced vaccine that can provide protection against the disease. The vaccine would be given to wildlife in an effort to prevent the spread of the disease to cattle [41].

Currently, treatment of bovine tuberculosis is not recommended due to its infectious nature. If an animal is found to be infected, it should be culled from the herd. However, there are some preventative measures available [68]. One way to ensure that cattle do not become infected is to eliminate any possible interaction with deer. The indirect contact is usually a result of cattle ingesting feed that has been contaminated by deer saliva. It is recommended that any feed for cattle be protected and stored away from deer. Other programs to control the deer population, such as hunting and banning feeding, have been implemented to decrease the density of deer and the population of affected deer [41]. There is long-term drug therapies that could, in principle, be used to treat the condition. Although the anti-tuberculosis drug Pyrazinamide is ineffective against *M. Bovis*, the use of isoniazid and rifampicin could be used effectively [97].

In Ethiopiaon government owned dairy farms, test and isolation of reactors combined with pasteurization of milk are the current undergoing control practices. However, these measures, as compared to the cattle population of the country, are found to be insignificant [26]. (In general terms, control measures in the traditional extensive production systems are more difficult and complex. In Ethiopia so far, control of BTB through the test-and-slaughter policy is not yet established. Most commonly culling of infected animals (Especially in government: owned farms) and improving sanitary and hygienic standards in other dairy farms is the actual control measure of BTB infection [98].

CONCLUSIONS

Mycobacterium bovis is the causative agent of bovine tuberculosis (BTB) and belongs to the *Mycobacterium tuberculosis* complex (MTBC) of bacterial strains. The most prominent member of the MTBC is *M.*

tuberculosis, the principle causative agent of tuberculosis in humans, causing each year more than 1.5 million deaths and having experienced a recent re-emergence through the advent of HIV/AIDS and the appearance of multi drug resistant strains. Moreover, wildlife reservoirs of *M. bovis* hamper disease eradication schemes in several countries. It also bears a zoonotic potential and it is of public health concern. It affects the national and international economy in different ways. The most obvious losses from BTB in cattle are direct productivity losses (Reduced benefit), which can be categorized into slaughter and "on-farm" losses. Slaughter losses comprise the cost of cattle condemnation and retention, with the loss from condemnation being essentially the purchased value of a slaughter animal and the loss from retention being a fraction of the value of a carcass. On-farm losses comprise the losses from decreased milk and meat production, the increased reproduction efforts and replacement costs for infected cattle. Therefore, based on the above conclusions the following recommendations have been forwarded:

- ✓ A great effort should be done for the diagnosis of the disease and provide useful information for use by public health and agricultural officials.
- ✓ Public health information campaigns are needed to raise community awareness about the risk of TB transmission through consumption of raw/undercooked meat.
- ✓ Organize regular capacity building through in-service training for both professionals (Technical training) and zoonotic diseases awareness training for non-professional personnel working in abattoirs should be recommended.
- ✓ Standardization of abattoir inspection protocols, enhanced training and proficiency testing of meat inspections and raising public awareness are recommended as essential and cost-effective interventions to improve meat inspection service in Ethiopia, with subsequent protection of consumers' health.
- ✓ Due attention should be given to this economic importance disease from the government and redress the concern to minimize its occurrence together with health professionals.
- ✓ A great interaction among the livestock owners, medical and veterinary personnel is the perquisites for the investigation of zoonotic importance of *M.bovis* and further investigations for minimizing its devastating effect in animals and humans.

REFERENCES

1. Terefe, D., 2012.Gross pathological lesions of bovine tuberculosis and efficiency of meat inspection procedure to detect-infected cattle in Adama municipal abattoir: Department of Pathology and Parasitology, College of Veterinary Medicine, Haramaya University, Ethiopia. J. Vet. Med. Anim. Health, pp: 49-53.
2. Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable, 2007. Veterinary Medicine: A text book of the diseases of cattle, horses, sheep, pigs and goats. 10thed. London: Saunders, pp: 1007-1014.
3. Alhaji, I., 2009. Mycobacteriological study of bovine granulomata in four Northern states of its environmental implications in the Niger Delta. Toxicol Environ Chem, 91: 619-25.
4. De la Rua-Domenech, R., 2006. Human Mycobacterium bovis infection in the United Kingdom: Incidence, risks, control measures and review of the zoonotic aspects of bovine tuberculosis. Tuberculosis (Edinb), 86: 77-109.
5. Muller, B.I., 2010. Molecular epidemiology and diagnosis of "Mycobacterium bovis" infections in African cattle, PhD Thesis, University of Basel, Faculty of Science. Official UR available at [:http://edoc.unibas.ch/diss/DissB_911].
6. Koro, F.K., E.F. Bouba, A.F. Ngatchou, S. Eyangoh and F. Etoa, 2013. First Insight into the Current Prevalence of Bovine Tuberculosis in Cattle Slaughtered in Cameroon: the Case of Main Abattoirs of Yaoundé and Douala, British Microbiology Research Journal, 3(3): 272-279.
7. Raghvendra, S.V., G.S. Arya, M.D. Hedaytullah, S.Tyagi, R. Kataria, S.J. Pachpute and A.P. Sharm, 2010. Clinical Aspects of Bovine Tuberculosis - A Chronic Bacterial Disease of Cattle: An Overview. International Journal of Phytopharmacology, India1 (2): 114-118.
8. Michel, A.L., B. Müller and P.D. Van Helden, 2010. Mycobacterium bovis at the animal–human interface: a problem, or not? Vet Microbiol., 140: 371-381.
9. Perry, B.D., T.F. Randolph, J.J. McDermott, K.R. Sones and P.K. Thornton, 2002. Investing in Animal Health Research to Alleviate Poverty. ILRI (International Livestock Research Institute): Nairobi, Kenya.
10. Boland, F., G. Kelly, M. Good and S. More, 2010. Bovine tuberculosis and milk production in infected dairy herds in Ireland. Prev. Vet. Med., 93: 153-161.

11. Grace, D., J. Gilbert, T. Randolph and E. Kang'ethe, 2012. The multiple burdens of zoonotic disease and an ecohealth approach to their assessment. Trop Anim. Health Prod., 44: 67-73.
12. Thoen, C.O., J.H. Steele and M.J. Gilsdorf, 2006. Mycobacterium bovis Infection in Animals and Humans. Wiley-Blackwell.
13. Thoen, C.O., P. LoBue, D.A. Enarson, J.B. Kaneene and I.N. de Kantor, 2009. Tuberculosis: a re-emerging disease of animals and humans. Veterinaria Italiana, 45: 135-81.
14. Waters, W., M.V. Palmer, T.C. Thacker, W.C. Davis, S., Sreevatsan and Coussens, 2010. Tuberculosis immunity: opportunities from studies with cattle. Clin Dev Immunol, 42: 76-85.
15. Ameni, G., K. Amenu and M. Tibbo, 2010. Bovine Tuberculosis: Prevalence and Risk Factor Assessment in Cattle and Cattle Owners in Wuchale-Jida District, Central Ethiopia. In Veterinary Medicine, Institute of Pathobiology, Faculty of Veterinary Medicine, Addis Abeba, Ethiopia.
16. Pirson, C., G.J. Jones, S. Steinbach, G.S. Besra and H.M. Vordermeier, 2012. Differential effects of Mycobacterium bovis - derived polar and apolar lipid fractions on bovine innate immune cells, Veterinary Research, 2012, 43:54 doi: 10.1186/1297-9716-43-54.
17. Ayele, W.Y., S.D. Neill, J. Zinsstag, M.G. Weiss and I. Pavlik, 2004. Bovine tuberculosis: an old disease but a new threat to Africa. International Journal of Tuberculosis and Lung Disease, 8: 924-937.
18. Iredia, C.H., O.O. Oguntibeju, H.A. Lewis and K. Mokwena, 2011. Trends and characteristics of patients admitted with musculoskeletal tuberculosis to referral hospitals from 2003 to 2008. Afr. J. Microbiol. Res., 5(5): 532-540.
19. Marcotty, T., F. Matthys, J. Godfroid, L. Rigouts, G. Ameni, N. Gey van Pittlus, R. Kazwala, J. Muma, P. van Helden, K. Walravens, C. de Geoghegan, D. Mbotha, M. Otte, K. Amenu, N. Samra, C. Botha, M. Ekron, A. Jenkins, F. Jori, N. Kriek, C. McCrindle, A. Michel, D. Morar, F. Roger, E. Thys and P. vanden Bossche, 2009. Zoonotic Tuberculosis and brucellosis in Africa: neglected zoonoses or minor public-health issues? The outcomes of a multi-disciplinary workshop. Ann. Trop. Med. Parasit, 103(5): 401-411.
20. World Health Organization (WHO), 2007. Global tuberculosis control: surveillance, planning and financing. Geneva, World Health Organization report, pp: 376.
21. Tschopp, R.J., Hattendorf, F. Roth, A. Choudhoury, A. Shaw, A. Aseffa and J. Zinsstag, 2012. Cost Estimate of Bovine Tuberculosis to Ethiopia, current topics in microbiology and immunology, Imperial College, London, UK, 365: 249-268.
22. Ameni, G., A. Regassa, T. Kassa and G. Medhin, 2001. Survey on bovine tuberculosis in cattle and its public health implications to cattle raising families in Wolaita Soddo, Southern Ethiopia. Ethiopian. J Anim Prod, 1: 55-62.
23. Asseged, B., A. Lubke-Becker, E. Lemma, K. Taddele and S. Britton, 2000. Bovine TB: a cross-sectional and epidemiological study in and around Addis Ababa. Bull. Anim. Heal. Prod. Afr, 48: 71-80.
24. Ameni, G. and A. Wudie, 2003. Preliminary study on bovine tuberculosis in Nazareth municipality abattoir of central Ethiopia. Bull. Anim. Heal. Prod. Afr, 51: 125-132.
25. Asseged, B., Z. Woldesenbet, E. Yimer and E. Lemma, 2004. Evaluation of Abattoir Inspection for the diagnosis of Mycobacterium bovis infection in cattle at Addis Ababa Abattoir. Tropical Animal Health and Production, 36: 537-546.
26. Bekele, M. and I. Belay, 2011. Evaluation of Routine Meat inspection procedures to detect Bovine Tuberculosis Suggestive Lesions in Jimma Municipal Abattoir, South West Ethiopia. School of veterinary medicine, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia, Global Veterinaria, 6(2): 172-179.
27. Biffa,D., A. Bogale and E. Skjerve, 2010. Diagnostic efficiency of abattoir meat inspection service in Ethiopia to detect carcasses infected with Mycobacterium bovis: Implications for public health: BMC Public Health, 10: 462.
28. Buddle, B.M., D.N. Wedlock and M. Denis, 2006. Progress in the development of tuberculosis vaccines for cattle and wildlife.Vet Microbiol, 112(2-4): 313-323.
29. Parkale, D.D. and A. Kulkarni, 2011. Bovine Tuberculosis and Its Control. Livestock Development Officer WRDDL, DIS, PUNE, 7: 1-9.
30. Vordermeier, M., G. Ameni, S. Berg, D. Bishop, B.D. Robertson, A. Aseffa, G. Hewinson and D.B. Young, 2012. The influences of cattle breed on susceptibility to bovine tuberculosis in Ethiopia. Comparative Immunology Microbiology and Infectious Diseases, 35(3): 227-232.

31. Kubica, T., R. Agzamova, A. Wright, G. Rakishev, S. Rüsch-Gerdes and S. Niemann, 2006. Mycobacteriumbovis Isolates with M. tuberculosis Specific Characteristics. Emerging Infectious Diseases • www.cdc.gov/eid, Vol., 12, No. 5, May 2006, pp: 763-765.
32. Brosch, R., S.V. Gordon, M. Marmiesse, P. Brodin, C. Buchrieser, K. Eiglmeier, T. Garnier, C. Gutierrez, G. Hewinson, K. Kremer, L.M. Parsons, A.S. Pym, S. Samper, D. van Soolingen and S.T. Cole, 2002. A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proceedings of the National Academy of Sciences, 99: 3684-3689.
33. Mostowy, S., D. Cousins, J. Brinkman, A. Aranaz and M.A. Behr, 2002. Genomic deletions suggest a phylogeny for the Mycobacterium tuberculosis complex. Journal of Infectious Diseases, 186, p. 74-80.
34. Rosevear, M and D. Urlich, 2010.Bovine Tb Strategy, Review of Costs. Specialists in Cost Benefit Analysis 42 Seaview Rd Paremata, Wellington, pp: 1-88.
35. Innes, P., 2008. Bovine Tuberculosis, Epidemiology, veterinary science. France, Omafra, pp: 1-2.
36. Neill, S.D., R.A. Skuce and J.M. Pollock, 2005. Tuberculosis--new light from an old window. J Appl Microbial, 98(6): 1261-9.
37. Animal Health Australia (AHA), 2009. Bovine Tuberculosis Case Response Manual, Managing an Incident of Bovine Tuberculosis: TB Case Response Manual Ed2 FINAL IanL (04-11-09).doc, pp: 1-9.
38. Dankner, W.M., N.J. Waecker, M.E. Essey, K. Moser, M. Thompson and C.E. Davis, 1993. Mycobacterium bovis infection in San Diego: a clinic epidemiologic study of 73 patients and a historical review of a forgotten pathogen. Medicine, 72: 11-37.
39. World Health Organization (WHO), 1994. Zoonotic tuberculosis (Mycobacterium bovis): a memorandum from WHO (With par, 8, Pp. ticipation of FAO). Bulletin of the World Health Organization, 72(6): 851-7.
40. LoBue, P.A., J.J. LeClair and K.S. Moser, 2004. Contact investigation for cases of pulmonary Mycobacterium bovis. Int. J. Tuberc. Lung. Dis., 8: 868-72.
41. Paylor, R., 2014. Bovine tuberculosis: Michigan State University Veterinary Student, Newsletter page 1-3-edited by Dr. Pam Mouser, Graduate Student.
42. Griffin, J.M., D.H. Williams, G.E. Kelly, T.A. Clegg, I. O'Boyle, J.D. Collins and S.J. More, 2005. The impact of badger removal on the control of tuberculosis in cattle herds in Ireland. Prev. Vet. Med., 67: 237-266.
43. Courtenay, O., L.A. Reilly, F.P. Sweeney, V. Hibberd, S. Bryan, A. Ul-Hassan, N. Newman, D.W. Macdonald, R.J. Delahay, G.L. Wilson and E. M.H. Wellington, 2006. Is Mycobacterium bovis in the environment important for the persistence of bovine tuberculosis? Biol Lett. USA. Sep, 22; 2(3): 460-462.
44. Karolemeas, K., T.J. McKinley, R.S. Clifton-Hadley, A.V. Goodchild, A. Mitchell, W.T. Johnston, A.J. Conlan, C.A. Donnelly and J.L. Wood, 2011. Recurrence of bovine tuberculosis breakdowns in Great Britain: Risk factors and prediction. Prev. Vet. Med. 2011 Jul, 16, p. 112-155.
45. Reviriego Gordejo, F.J. and J.P. Vermeersch, 2006. Towards eradication of bovine tuberculosis in the European Union. Vet. Microbiol., 112: 101-109.
46. Radunz, B., 2006. Surveillance and risk management during the latter stages of eradication: experiences from Australia. Vet. Microbiol., 112: 283-290.
47. Torgerson, P. and D. Torgerson, 2009. Benefits of stemming bovine TB need to be demonstrated. Nature, 457: 657.
48. Bennett, R.M., 2009. Farm costs associated with premovement testing for bovine tuberculosis. Vet. Rec., 164: 77-79.
49. Amanfu, W., 2006. The situation of tuberculosis and tuberculosis control in animals of economic interest. Tuberculosis, 86: 330-335.
50. de Kantor, I.N., M. Ambroggi, S. Poggi, N. Morcillo, M.A. Da Silva Telles, M. Osorio Ribeiro, M.C. Garzon Torres, C. Llerena Polo, W. Ribon, V. Garcia, D. Kuffo, L. Asencios, L.M. Vasquez Campos, C. Rivas, J.H. de Waard, 2008. Human Mycobacterium bovis infection in ten Latin American countries. Tuberculosis, 88: 358-365.
51. Cosivi, O., J.M. Grange, C.J. Daborn, M.C. Raviglione, T. Fujikura, D. Cousins, R.A. Robinson, H.F. Huchzermeyer, I. de Kantor and F.X. Meslin, 1998. Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerg. Infect. Dis., 4: 59-70.
52. WHO. http://www.who.int/entity/zoonoses. Last updated; 2009, last accessed, 10/06/2009
53. Diguimbaye, C., E. Schelling, G.E. Pfyffer, F. Baggi, R. Ngandolo, G. Ndoutamia, M. Tanner and J. Zinsstag, 2004. First isolation of tuberculous mycobacteria in man and animals in Chad. Med. Trop., 64: 482-485.

54. Oloya, J., J.B. Muma, J. Opuda-Asibo, B. Djønne, R. Kazwala and E. Skjerve, 2007. Risk factors for herd-level bovine-tuberculosis seropositivity in transhumant cattle in Uganda. Prev. Vet. Med., 80: 318-329.
55. Srivastava, K., D.S. Chauhan, P. Gupta, H.B. Singh, V.D. Sharma, V.S. Yadav, Sreekumaran, S.S. Thakral, J.S. Dharamdheeran, P. Nigam, H.K. Prasad, V.M. Katoch, 2008. Isolation of Mycobacterium bovis & M. tuberculosis from cattle of some farms in North India—possible relevance in human health. Indian J. Med. Res., 128: 26-31.
56. de Kantor, I.N. and V. Ritacco, 2006. An update on bovine tuberculosis programmes in Latin American and Caribbean countries. Vet. Microbiol., 112: 111-118.
57. Munyeme, M., J.B. Muma, K.L. Samui, E. Skjerve, A.M. Nambota, I.G. Phiri, L. Rigouts and M. Tryland, 2009. Prevalence of bovine tuberculosis and animal level risk factors for indigenous cattle under different grazing strategies in the livestock/wildlife interface areas of Zambia. Trop. Anim. Health Prod., 41: 345-352.
58. Ameni, G., K. Amenu and M. Tibbo, 2003. Bovine tuberculosis: prevalence and risk factor assessment in cattle and cattle owners in Wuchale-Jida District, Central Ethiopia. Int. J. Appl. Res. Vet. Med., 1: 17-26.
59. Proano-Perez, F., L. Rigouts, J. Brandt, P. Dorny, J. Ron, M.A. Chavez, R. Rodriguez, K. Fissette, A. Van Aerde, F. Portaels, W. Benitez-Ortiz, 2006. Preliminary observations on Mycobacterium spp. in dairy cattle in Ecuador. Am. J. Trop. Med. Hyg., 75: 318-323.
60. Tadayon, K., N. Mosavari, F. Sadeghi and K.J. Forbes, 2008. Mycobacterium bovis infection in Holstein Friesian cattle, Iran. Emerg. Infect. Dis., 14: 1919-1921.
61. Somali Medical Forums (SMF), 2009. Topic: Epidemiology of Mycobacterium, causative agents of Tuberculosis. Biological Properties of Representatives Mycobacterium Tuberculosis. Pathogenesis, Laboratory Diagnostics, Control and Prophylaxis of Tuberculosis, pp: 1-5.
62. Geijtenbeek, T.B. and S.I. Gringhuis, 2009. Signalling through C-type lectin receptors: shaping immune responses. Nat. Rev. Immunol., 9: 465-479.
63. Sinsimer, D., G. Huet, C. Manca, L. Tsenova, M.S. Koo, N. Kurepina, B. Kana, B. Mathema, S.A. Marras, B.N. Kreiswirth, C. Guilhot and G. Kaplan, 2008. The phenolic glycolipid of Mycobacterium tuberculosis differentially modulates the early host cytokine response but does not in itself confer hyper virulence. Infect Immun, 76: 3027-3036.
64. Hope, J.C., A.O. Whelan, R.G. Hewinson, M. Vordermeier and C.J. Howard, 2003.Maturation of bovine dendritic cells by lipopeptides. Vet Immunol Immunopathol., 95: 21-31.
65. Andersen, C.S., E.M. Agger, I. Rosenkrands, J.M. Gomes, V. Bhowruth, K.J. Gibson, R.V. Petersen, D.E. Minnikin, G.S. Besra and P. Andersen., 2009. A simple mycobacterial monomycolated glycerol lipid has potent immunostimulatory activity. J. Immunol., 182: 424-432.
66. Reed, M.B., P. Domenech, C. Manca, H. Su, A.K. Barczak, B.N. Kreiswirth, G. Kaplan and C.E. Barry, 2004. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature, 431: 84-87.
67. Koehler, A., 2010. Mycobacterium bovis. Microbe wiki, n.d. Web. 18 Feb. 2010. Available at [http://microbewiki.kenyon.edu/index.php/Mycobacterium_bovis] (accessed on March, 2014).
68. Peters, D., 2010. Farm Animals, Signs and Symptoms of Tuberculosis in Cattle.UK, pp: 1-8.
69. Office International des Epizooties (OIE), 2009, 'Bovine tuberculosis', in Manual of diagnostic tests and vaccines for terrestrial animals. Viewed 11 July 2013, from [http:// www.oie.int/ fileadmin/ Home/ eng/ Health_standards/tahm/2.04.07].
70. Quinn, P.J. and B.K. Markey, 2003. Concise review of Veterinary Microbiology. UK: Blackwell Publishing, pp: 34-37.
71. Quinn, P.J., 1994. Clinical Veterinary Microbiology. Spain: Mosby, pp: 158-169.
72. Patterson, J. and D. Grooms, 2000. Diagnosis of bovine tuberculosis: Gross necropsy, histopathology and acid fast staining. Michigan State University Extension, 35: 1-2.
73. Scanlar, M.C., 1988. Introduction of Veterinary Bacteriology. USA: Lowa State University Press/ Ames, pp: 118-123.
74. Bisping, W. and G. Amtsberg, 1988. Color Atlas for the Diagnosis of Bacterial pathogens in animals. Germany: Paul Parey Scientific Publishers, pp: 108-118.

75. Katial, K.R., J. Hershey, P.T. Seth, T.J. Belisle, J.P. Brannan, S.J. Spancer and M.J. Engler, 2001. Cell-mediated immune response to tuberculosis antigens: Comparison of skin testing and measurement of in vitro gamma interferon production in whole-blood culture. Clinical and diagnostic lab Immunology, 8: 329-345.
76. Tizard, L.R., 2004. Veterinary immunology, an introduction. 7th ed. Texas A and M University: Sounders, Elseveir, pp: 343-350.
77. Tewodros, F. and L. Girja, 2012. Diagnostic Techniques of Bovine Tuberculosis: A Review. African Journal of Basic & Applied Sciences, 4(6): 192-199.
78. Espinosa De los monteros L.E., J.C. Galan, M. Gutierrez, S. Samper, J.F. Garcia marin, C. Martin, L. Dominguez, L. De Rafael, F. Baquero, E. Gomez-mampaso and J. Blazquez, 1998. Allele-specific PCR method based on pncA and oxyR sequences for distinguishing Mycobacterium bovis from M. tuberculosis: intraspecific M. bovis pncA sequence polymorphism. J. Clin. Microbiol., 36: 239-242.
79. Kamerbeek J., L. Schouls, A. Kolk, M. Van Agterveld, D. Van soolingen, S. Kuijper, A. Bunschoten, H. Molhuizen, R. Shaw, M. Goyal & J. Van Embden, 1997. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J. Clin. Microbiol., 35: 907-914.
80. Noredhoek, G.T., J.D.A. Van Embden and A.H.J. Kolk, 1996. Reliability of nucleic acid amplification for detection of Mycobacterium tuberculosis: an international collaborative quality control study among 30 laboratories. J. Clin. Microbiol., 34: 2522-2525.
81. Miller, J., A. Jenny, J. Rhgyan, D. Saari and D. Saurez, 1997. Detection of Mycobacterium bovis in formalinfixed, paraffin-embedded tissues of cattle and elk by PCR amplification of an IS6110 sequence specific for M. tuberculosis complex organisms. J. Vet. Diagn. Invest., 9: 244-249.
82. Miller, J., A. Jenny and J. Payeur, 2002. Polymerase chain reaction detection of Mycobacterium bovis and M. avium organisms in formalin-fixed tissues from culture-negative organisms. Vet. Micro., 2328: 1-9.
83. Durr, P.A., R.S. Clifton-Hadley and R.G. Hewinson, 2000. Molecular epidemiology of bovine tuberculosis. II. Applications of genotyping. Rev. sci. tech. Off. int. Epiz., 19: 689-701.
84. Durr, P.A., R.G. Hewinson and R.S. Clifton-Hadley, 2000. Molecular epidemiology of bovine tuberculosis. I. Mycobacterium bovis genotyping. Rev. Sci. Tech. Off. int. Epiz., 19: 675-688.
85. Skuce, R.A., D. Brittain, M.S. Hughes and S.D. Neill, 1996. Differentiation of Mycobacterium bovis isolates from animals by DNA typing. J. Clin. Microbiol., 38, 2469-2474.
86. O'Brian, R., B.S. Danilowicz, L. Bailey, O. Flynn, E. Costello. D. O'Grady and M. Rodgers, 2000. Characterisation of the Mycobacterium bovis restriction fragment length polymorphism DNA probe pUCD and performance comparison with standard methods. J. Clin. Microbiol., 38: 3362-3369.
87. Cousins, D.V., R.A. Skuce, R.R. Kazwala and J.D.A. Van Embden, 1998. Towards a standardized approach to DNA fingerprinting of Mycobacterium bovis. Int. J. Tuberc. Lung Dis., 2: 471-478.
88. Garnier, T., K. Eiglmeier, J.C. Camus, N. Medina, H. Mansoor, M. Pryor, S. Duthoy, S. Grondin, C. Lacroix, C. Monsempe, S. Simon, B. Harris, R. Atkin, J. Doggett, R. Mayes L. Keating, P.R. Wheeler, J. Parkhill, B.G. Barrell, S.T. Cole, S.V. Gordon and R.G. Hewinson, 2003. The complete genome sequence of Mycobacterium bovis. Proc. Natl Acad. Sci. USA, 100(13): 7877-7882.
89. Zinsstag, J., E. Schelling, F. Roth and R.R. Kazwala, 2006. Economics of bovine tuberculosis: Mycobacterium bovis Infection in Animals and Humans. 2nd ed. Iowa State University: Press, Ames, Iowa, USA, pp: 68-83.
90. Munagandu, H.M., V.M. Siamudaala, A. Nambota, J.M. Bwalya, M. Munyeme and A.S. Mweene, 2006. Disease constraints for utilization of the Africanbuffalo (Syncerus caffer) on game ranches in Zambia. Jpn. J. Vet. Res., 54: 3-13.
91. Center for Disease Control and Prevention (CDC), 2011. Mycobacterium bovis (bovine tuberculosis) in Humans CDC Fact Sheet: Atlanta, Georgia, USA, Center for Disease Control and Prevention: available at [http:// www.cdc.gov/ tb/ publications/ factsheets/ general/mbovis.pdf].
92. Majoor, C.J., C. Magis-Escurra, J. van Ingen, M.J. Boeree and D. van Soolingen, 2011. Epidemiology of Mycobacterium bovis Disease in Humans, the Netherlands, 1993-2007: Emerg Infect Dis., 17: 457-63.
93. Good, M. and A. Duignan, 2011. Perspectives on the History of Bovine TB and the Role of Tuberculin in Bovine TB Eradication. Vet Med Int, pp: 1-11.

94. Grange, J.M., 2001. Mycobacterium bovis infection in human beings: Tuberculosis, USA, 81: 71-7.
95. Ashford, D.A., E. Whitney, P. Raghunathan and O. Cosivi, 2001. Epidemiology of selected mycobacterium that infect humans and other animals. Review of Science and Technology, Office International des Epizooties, pp: 23-54.
96. Regassa, A., 2005. Study on Mycobacterium bovis in animals and human in and around Fiche, North Shewa zone, Ethiopia. [MSc. Thesis.] Faculty of Veterinary Medicine, Addis Ababa University, Debre-Zeit, Ethiopia.
97. Thibodeaux, W., 2014. What is bovine tuberculosis? 1999-2014 Demand media, Inc, UK. pp: 1-5.
98. Shitaye, J.E., W. Tsegaye and I. Pavlik, 2007. Bovine tuberculosis infection in animal and human populations in Ethiopia: a review, Veterinarni Medicina, 52(8): 317-332.